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Research Article

Hydrophobically modified polyacrylamide block copolymers for fast, high-resolution DNA sequencing in microfluidic chips

By using a microfluidic electrophoresis platform to perform DNA sequencing, genomic information can be obtained more quickly and affordably than the currently employed capillary array electrophoresis instruments. Previous research in our group has shown that physically cross-linked, hydrophobically modified polyacrylamide matrices separate dsDNA more effectively than linear polyacrylamide (LPA) solutions. Expanding upon this work, we have synthesized a series of LPA-co-dihexylacrylamide block copolymers specifically designed to electrophoretically sequence ssDNA quickly and efficiently on a microfluidic device. By incorporating very small amounts of *N,N*-dihexylacrylamide, a hydrophobic monomer, these copolymer solutions achieved up to ~10% increases in average DNA sequencing read length over LPA homopolymer solutions of matched molar mass. Additionally, the inclusion of the small amount of hydrophobe does not significantly increase the polymer solution viscosities, relative to LPA solutions, so that channel loading times between the copolymers and the homopolymers are similar. The resulting polymer solutions are capable of providing enhanced sequencing separations in a short period of time without compromising the ability to rapidly load and unload the matrix from a microfluidic device.

Keywords:
DNA sequencing / Linear polyacrylamide / Microchip electrophoresis / Polymer solutions / Viscosity
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1 Introduction

The need for rapid, accurate, and affordable methods to analyze and sequence human genomic DNA has spurred technological advancements in an effort to realize personalized healthcare. The development of capillary array electrophoresis (CAE) systems, for example, allowed for the complete sequencing of the human genome ahead of schedule [1, 2]. Optimization of these systems and materials have demonstrated the capability to sequence up to 1300 bases in approximately 2 h [3], and commercial instruments can regularly achieve read lengths on the order of 700–1000 bases. In order to sequence repeat-rich genomes, these long read lengths are necessary [4, 5]. And although genome centers utilizing many CAE systems in parallel are capable of analyzing very large amounts of genomic material, the costs in terms of money and time for CAE are too high for widespread use of complete genome sequencing for routine medical care.

To reduce the overall cost of sequencing DNA, the current trend in Sanger-based technologies is shifting toward utilizing microfluidic devices for both sample preparation and DNA separation by electrophoresis [6–12]. Integrated microfluidic sequencing systems were pioneered by the Mathies group [13] and further sequencing studies have shown that on single-channel chips, up to 600 bases can be sequenced in under 7 min [14], and 96-microchannel array chips are capable of sequencing an excess of 100 000 bases/h when using post-purified and amplified DNA samples [15]. This combination of increased sequencing speed,

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Abbreviations: CAE, capillary array electrophoresis**; DHA,** *N,N*-dihexylacrylamide**; HMPAM,** hydrophobically modified polyacrylamide**; IPA,** isopropyl alcohol**; LPA,** linear polyacrylamide**; pHEA,** poly-*N*-hydroxyethylacrylamide

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along with reduced sample and reagent requirements and on-chip integration of sample preparation steps will lower the overall cost to sequence a genome and advance the development of point-of-care field devices for testing patients and detecting various harmful biological agents.

Linear polymer solutions are commonly used for DNA separations on capillaries and microfluidic chips because they can easily be loaded into and removed from the separation channel [16–20]. To achieve read lengths of 500–700 bases on microfluidic devices, polymers in excess of 1 MDa and polymer solution concentrations of ~4% w/v are typical, which can lead to very viscous matrices. Increasing the molar mass of the separation polymer can increase DNA sequencing read lengths [21], however, this also increases the solution viscosity and therefore the amount of time required to load the separation matrix into a microchannel [22–24].

Here, we describe the synthesis of hydrophobically modified block copolymers composed primarily of a hydrophilic acrylamide backbone structure with small, randomly placed blocks of *N,N*-dihexylacrylamide (DHA), and the formulation of these materials into DNA sequencing matrices [25–27]. We show that with the addition of only ~0.1 mol% of the hydrophobic monomer, this system can give improvements of up to 10% in DNA sequencing read length over linear polyacrylamide (LPA) homopolymers of equivalent molar mass. This improvement in performance is attributed to the physical cross-linking that takes place between the hydrophobic blocks in the copolymers, forming a more robust network structure. However, the physical associations can be broken and reformed through the application of shear forces when loading the copolymer into a microfluidic channel, so that the matrix loading time is essentially unaffected by the presence of the hydrophobic blocks in the copolymer [26, 28].

2 Materials and methods

2.1 Monomer synthesis

The hydrophobic DHA monomer utilized in this study was synthesized in our lab as described previously [27], using a process similar to one originally described by McCormick *et al*. [29]. The reagents dihexylamine, triethylamine, acryloyl chloride, and methylene chloride were all purchased from Sigma-Aldrich (Milwaukee, WI, USA) and were used as received. The dihexylamine was initially dissolved at 10 mM in 120 mL of methylene chloride along with an equimolar concentration of triethylamine, and added into a round-bottom flask, which was placed in an ice bath throughout the reaction process. In a separate beaker, acryloyl chloride was dissolved at 10 mM into 120 mL of methylene chloride and subsequently added drop-wise into the amine solution, at a rate of approximately 0.5 mL/min, under a nitrogen atmosphere. Once the acryloyl chloride solution was completely incorporated, the mixture was allowed to react for an additional hour before purification. The reaction product was purified by performing three aqueous extractions in series utilizing 1 M HCl, 0.5 M NaOH, and finally DI water. Following the final extraction, the methylene chloride phase was removed with a rotary evaporator attached to a dry ice/acetone/methanol cold trap, leaving behind the pure DHA monomer.

2.2 Polymer and copolymer synthesis and characterization

All of the polymerizations took place under nitrogen in 500 mL three-neck flasks attached to a temperature-controlled recirculating water bath. The LPA and poly-*N*-hydroxyethylacrylamide (pHEA) utilized in this study were both synthesized *via* standard aqueous-phase free-radical polymerization methods. For the LPA homopolymers, acrylamide monomer (Amresco, Solon, OH, USA) was dissolved at 4% w/w in DI water along with approximately 1–3% w/w of isopropyl alcohol (IPA) (Fisher Scientific, Waltham, MA, USA), which acts as a chain transfer agent, to obtain the desired average molar mass. pHEA was synthesized using a similar procedure where HEA monomer (Cambrex, East Rutherford, NJ, USA) was dissolved at 2% w/w in DI water along with 0–0.2% w/w of IPA. For both reactions, the solution was degassed for at least 30 min with nitrogen and heated to 50°C prior to initiation. The polymerizations were initiated with 0.004–0.005 g of 4′-azobis(4-cyanovaleric acid) *per* 100 g of monomer solution, and allowed to react for 6 h. The resulting polymers were dialyzed in 100 kDa molecular weight cutoff membranes against DI water for 2 wk, with 15–20 water changes to remove any unreacted monomer and low molar mass material. Following dialysis, the polymers were frozen at −80°C and recovered by lyophilization.

Hydrophobically modified polyacrylamide (HMPAM) block copolymers cannot be synthesized by standard aqueous-phase free-radical polymerization techniques. The *N,N*-dialkylacrylamide monomers are not soluble in aqueous environments, therefore micellar free-radical polymerization was used to achieve the desired structure. A second phase consisting of SDS micelles was used to incorporate the hydrophobic monomer into the copolymer backbone and also allows the average number of hydrophobic molecules included into each block to be controlled [25, 30]. The LPA-co-DHA used for sequencing was synthesized by dissolving acrylamide monomer at 4% w/w in DI water along with 0.1 mol% of DHA monomer, and approximately 1–3% w/w chain transfer agent, IPA, to obtain the desired molecular weight. Approximately, 0.46 g of SDS (Sigma, St. Louis, MO, USA) was added *per* 100 g of monomer solution to obtain a desired average hydrophobic block size of 5 units. Prior to initiation, the solution was allowed to mix for 30 min, degassed for an additional 30 min with nitrogen and heated to 50°C. The polymerization was initiated with 0.005 g of 4′-azobis(4-cyanovaleric acid) *per* 100 g

of monomer solution, and allowed to react for 5–6 h. The resulting polymers were precipitated with acetone, to help remove the SDS present in the reaction mixture, redissolved in DI water, and dialyzed in 100 kDa molecular weight cutoff membranes against DI water for 2 wk with 15–20 water changes to remove any unreacted monomer, SDS, and low molar mass material. Following dialysis, the copolymers were frozen at −80°C and recovered by lyophilization.

To determine the average polymer molar mass, polydispersity index, and root mean square radius of gyration, the polymers were fractionated *via* tandem gel permeation chromatography with a Waters 2690 Alliance Separations Module (Waters, Milford, MA, USA) outfitted with Shodex OHpak SB-806 HQ, SB-804 HQ, and SB-802.5 HQ columns (New York, NY, USA) connected in series. The fractionated polymer solution was sent through a DAWN DSP Laser Photometer Multi-angle laser light scattering instrument followed by an Optilab DSP refractive index detector (both instruments from Wyatt Technology, Santa Barbara, CA, USA). The resulting data were analyzed using ASTRA (Wyatt Technology) based on a known sample concentration, 100% mass recovery, and known system constants. Both LPA and pHEA were dissolved at 1 mg/mL in a buffer containing 0.1 M NaCl, 50 mM NaH_2PO_4, and 200 ppm NaN_3, while the LPA-co-DHA was dissolved at 5 mg/mL in a 33% methanol buffer, due to the moderately hydrophobic nature of the copolymer.

2.3 Viscosity and microfluidic chip loading measurements

Rheological tests were performed on a Paar Physica MCR 300 Rheometer (Ashland, VA, USA) outfitted with a 50 mm, 2° cone-and-plate fixture held at 25°C by a recirculating water bath (Julabo USA, Allentown, PA, USA) connected to a TEK 150PA-C model Peltier controller. Approximately, 1.14 mL of the polymer solution was injected between the cone and plate fixture and analyzed using a shear rate sweep from 0.1 to 1000 s^{-1} and a shear stress sweep from 0.01 to 100 Pa utilizing a logarithmic ramp and collecting seven points *per* decade. The resulting data were analyzed to remove any points below the torque limit of the machine and overlaid to create a complete series of data from ∼0.01 to 1000 s^{-1}.

Load times of the polymer solutions into the 8 cm glass microfluidic chips were determined using a modified hydraulic press applying ∼200 psi of pressure *via* an attached nitrogen tank. The amount of time required to fill up the entire chip with the polymer solution was recorded for each of the sequencing polymer matrices utilized in this study.

2.4 Microchip electrophoresis

The M13 sequencing ladder and ssDNA ET-900 Ladder (both from Amersham GE Healthcare, Piscataway, NJ, USA) were analyzed on a microchip electrophoresis system custom-built in-house, which is capable of multi-color LIF detection. This system has been previously described in detail by Chiesl *et al.* [27], and consists of electrical and optical subsystems that can be used in conjunction with a temperature-control plate capable of maintaining the microchips at 50°C for sequencing experiments.

The T8050 borosilicate glass microfluidic chips used in this study were purchased from Micronit Microfluidics (Enschede, The Netherlands) and consist of a single channel that has an effective DNA separation length of 7.5 cm. Each channel is 50 μm wide, 20 μm deep, and utilizes a 100 μm offset T injection scheme. Prior to use, the channels were dynamically coated with a 0.10% w/w solution of pHEA to reduce electroosmotic flow and bioanalyte adsorption onto the channel walls [31, 32].

The DNA separation experiments were performed using LPA and LPA-co-DHA solutions of 2–4% w/w dissolved in 1 × TTE (49 mM Tris, 49 mM TAPS, and 2 mM EDTA) with 7 M urea. Each test consisted of a 60 s pre-electrophoresis of the primary separation channel at an electric field of ∼187 V/cm. This was followed by a 40 s injection of the sample at 400 V/cm. The plug of DNA was then separated at 187 V/cm in the primary channel with pullback fields of 150 V/cm being applied to the sample and sample waste wells to prevent leakage of sample into the separation channel. The resulting data were analyzed using NNIM Basecaller (NNIM, LLC, Salt Lake County, UT, USA) and Sequencher V 4.05 (Gene Codes, Ann Arbor, MI, USA).

2.5 Single-molecule fluorescent imaging of electrophoresing DNA

Individual molecules of λ-DNA (Invitrogen, San Diego, CA, USA) fluorescently labeled with YOYO-1 were imaged *via* a lab-built epifluorescence video microscopy system as previously described [28, 33]. The voltage (∼100 V/cm) for electrophoresis of the DNA was obtained using a modified Micronit Microfluidics electrophoresis kit. The imaging setup was composed of a Nikon TE2000 U inverted epifluorescence microscope (Nikon Instruments, Melville, NY, USA) with a Nikon CFI 100 × /N.A. 1.4 oil immersion objective. Fluorescence was obtained with a 100 W Hg broadband lamp focused through a blue light excitation filter cube (460–500 nm) (Chroma Technology, Rockingham, VT, USA) and reflected off of a dichroic beam splitter through the objective. The resulting fluorescence returns through the dichroic beam splitter and a 510 nm long pass filter. The signal is amplified with a VS4-1845 Generation 3 image intensifier (Videoscope International, Dulles, VA, USA) and collected on a 0.5″ CCD TM-6710-CL camera (JAI Pulnix, Sunnyvale, CA, USA). The videos of the DNA molecules were collected at 30 frames/s onto a computer utilizing XCAP-STD software and a PIXCI control board (EPIX, Buffalo Grove, IL, USA).

3 Results and discussion

3.1 DNA sequencing with HMPAMs

HMPAMs were originally developed in our group by Chiesl *et al*. to be utilized as a sort of hydrophobic ''guard column'' for the removal of proteins from crude biological samples while allowing DNA to pass through unhindered [27]. During this study it was observed that dsDNA was separated with greater resolution using HMPAMs compared with an LPA of matched molar mass [28]. Here, we expand upon that work by analyzing the DNA sequencing capability of specially tailored hydrophobically modified copolymers when compared with equivalent molar mass homopolymers of LPA. A series of copolymers were synthesized with average molar masses of 1.4, 2.8, and 7.3 MDa, each containing ~0.10 mol% of DHA; and polymer solutions were formulated at concentrations ranging from 2 to 4% w/w in 1 × TTE aqueous buffers with 7 M urea. Copolymers containing ~0.10 mol% DHA were synthesized for this study because Chiesl *et al*. showed that there is no discernable improvement in dsDNA separation resolution obtained in HMPAMs by exceeding this value. Additionally, increasing the hydrophobe content increases the viscosity of the copolymer separation matrix, making it more difficult to quickly load and unload the copolymer solutions from the microfluidic channels [28]. Matched molar mass LPAs were synthesized at 1.4 and 2.8 MDa and 4% w/w solutions of the polymers were formulated for comparison with the copolymer solutions. The physical properties of the polymers utilized in this study are given in Table 1. The sequencing performance of the LPA and LPA-co-DHA polymer solutions were determined using an ssDNA M13 DNA sequencing standard in glass microfluidic chips dynamically coated with pHEA. The average read length was obtained by taking the average of four sequencing runs with the 4% solutions and the average of two sequencing runs with the 2 and 3% solutions (at 98.5% accuracy when compared with the known M13 DNA sequence).

As can be seen in Table 2, the average sequencing read length increases significantly as the polymer concentration is changed from 2 to 4%, and the longest sequencing lengths are obtained in the 4% solutions. It was also observed that the maximum average sequencing length increased slightly from 554 to 583 bases with the increase in molar mass from 1.4 to 7.3 MDa with the longest observed run yielding over 600 bases, as shown in Fig. 1. This small variation in read length with such a large increase in polymer molar mass is beneficial from a quality control perspective. If a small variation in molecular weight has a large impact on the sequencing performance, the synthesis requirements become much more stringent, and batches of expensive polymer could easily be wasted. If the final average molar mass does not significantly impact the sequencing capability, less polymer would be wasted, and less rigorous synthesis protocols would be necessary.

Interestingly, an ~10% increase in average sequencing read length (554 *versus* 508 bases) was obtained with the 1.4 MDa LPA-co-DHA when compared with an LPA at the same molar mass. The improvement in sequencing read length appears to decrease at the higher molecular weight, as the 2.8 MDa LPA-co-DHA copolymer provides an average read length only ~4% greater than the 2.8 MDa LPA (561 *versus* 541 bases). The 4% solutions tested in this study achieved their separations rapidly, taking only 10–11.5 min to sequence 550–600 bases. Previously published sequencing results on microfluidic devices have typically ranged from 15 to 30 min, with recent chip-based sequencing data from Fredlake *et al*. showing 600-base reads that are complete within 6.5 min [6, 7, 14].

Table 1. Properties of the acrylamide-based polymers and copolymers utilized in this microfluidic chip DNA sequencing study

Polymer	Hydrophobe concentration (mol%)	M_w (MDa)	R_g (nm)	PDI
LPA	0	1.5	58	1.7
	0	2.8	79	1.7
LPA-co-DHA	0.1	1.4	52	1.6
	0.1	2.8	63	2.0
	0.1	7.3	77	1.4
pHEA	0	3.7	76	1.3

Table 2. ssDNA sequencing results obtained with LPA-co-DHA copolymers and LPA homopolymers of matched molar mass[a)]

Sequencing polymer	High	Low	Average
~1.4 MDa LPA-co-DHA, ~0.1 mol% DHA			
2%	40	22	**31 ± 13**
3%	426	309	**368 ± 83**
4%	568	535	**554 ± 16**
~2.8 MDa LPA-co-DHA, ~0.1 mol% DHA			
2%	414	287	**351 ± 90**
3%	493	429	**461 ± 45**
4%	576	533	**561 ± 20**
~7.3 MDA LPA-co-DHA, 0.1 mol% DHA			
2%	407	319	**363 ± 62**
3%	508	456	**482 ± 37**
4%	**606**	568	**583 ± 19**
~1.4 MDa LPA			
4%	524	495	**508 ± 12**
~2.8 MDa LPA			
4%	560	529	**541 ± 15**

a) Experimental conditions: 7.5-cm effective length glass microfluidic chip, 50°C, 187 V/cm separation field strength, $n = 4$ for 4% w/w solutions, $n = 2$ for 2 and 3% w/w solutions, results at 98.5% accuracy.

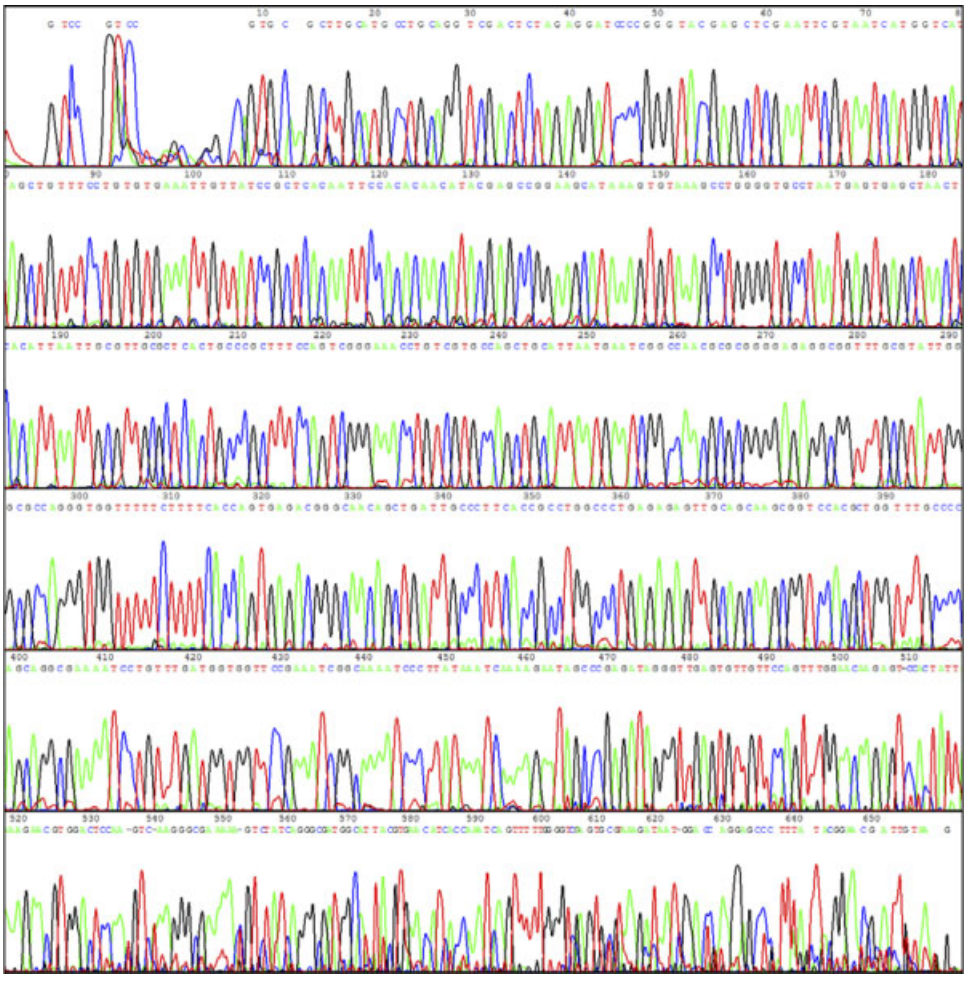


Figure 1. Electropherogram of an M13 ssDNA sequencing standard where 606 bases were successfully sequenced on a 7.5 cm effective length glass microfluidic chip using a 4% w/w 7.3 MDa LPA-co-DHA copolymer. (Experimental conditions: 50°C, 187 V/cm separation field strength, results at 98.5% accuracy.)

We hypothesize that the increase in sequencing read length obtained in the HMPAM copolymer networks is due to the intermolecular and intramolecular physical cross-linking that occurs between the hydrophobic blocks on the copolymer chains. This effect is believed to allow HMPAMs to mimic a larger molar mass homopolymer solution, because the strong physical associations between the polymer strands create a more robust network, resulting in more resistance to the migration of large DNA molecules, and hence longer average sequencing read lengths. This also explains the diminishing returns we observe in DNA sequencing performance of the copolymer in comparison to the homopolymer as the molecular weight increases, because the ability of a polymer solution to sequence DNA does not improve indefinitely with an increase in polymer molar mass.

3.2 Rheological characterization

The ability to reduce the time required for each sample analysis and therefore the overall cost of sequencing DNA is almost as important as increasing the number of bases that can be sequenced *per* lane in a microfluidic device. Hydrophobic block copolymers typically have higher viscosities than their homopolymer counterparts due to the impact of physical cross-linking [25], which could be a hindrance when using these materials in microfluidic devices. However, these polymer matrices require extremely small amounts of hydrophobe (0.1%) to achieve the desired increase in sequencing length over their homopolymer counterparts, and thus, have very similar channel loading times as LPA polymers with the same molar mass.

As shown in Fig. 2B, the average loading time for a 4% 1.4 MDa LPA solution was ~1 min. For an LPA-co-DHA copolymer solution with the same molar mass, the loading time was increased by only ~15 s. This is largely attributed to the small amount of hydrophobe present in the copolymer, as well as the ability of the physical cross-links between the hydrophobic groups to break and re-form when pressure is applied and removed, and the large amount of shear thinning observed with these polymer solutions. Figure 2A shows the pronounced shear thinning typically observed with these sequencing matrices, which occurs when the

entangled chains align themselves in the direction of the fluid flow. This phenomenon is observed when the solutions are placed under a large amount of shear [26, 28], such as when the solutions are loaded into the microfluidic channels under high pressure. The combination of these factors results in polymer solutions that provide very high-resolution separations over a short distance, and can be loaded and unloaded from a microfluidic device quickly and easily.

3.3 Mobility measurements correlated with single-molecule DNA imaging

DNA mobility measurements coupled with single-molecule fluorescent DNA imaging techniques were used to investigate the DNA migration mechanisms that might contribute to the observed sequencing results. The electrophoretic mobilities of ssDNA molecules are plotted against DNA fragment size for separations in 4% w/w polymer solutions in Fig. 3. The slopes of the linear regions of the mobility plots, which indicate when the DNA is primarily electrophoresing *via* unoriented biased reptation [34], were determined by fitting a power-law to the data with a minimum R^2 value of 0.999. The slopes of the 4% 1.4 MDa LPA-co-DHA and LPA polymer solutions were −0.58 and −0.56, respectively, and the slopes of the 4% 2.8 MDa LPA-co-DHA and LPA polymer solutions were −0.62 and −0.61, respectively. This decrease in DNA mobility is likely a result of the increased number entanglements created by the larger molar mass polymers as well as the physical cross-links between the hydrophobic groups. The DNA must push through this robust network of polymer strands, resulting in lower electrophoretic mobilities, and an increase in read lengths due to the greater number of interactions the DNA will have with the sieving matrix over a given distance.

To help confirm these findings, single-molecule DNA imaging was used to observe the predominant migration mechanisms of double-stranded λ-DNA when electrophoresing through the 4% w/w 1.4 MDa LPA and LPA-co-DHA polymer solutions. These polymers were selected for comparison because they had the most significant variation in DNA sequencing capability, and would present the greatest opportunity for observing differences in migration

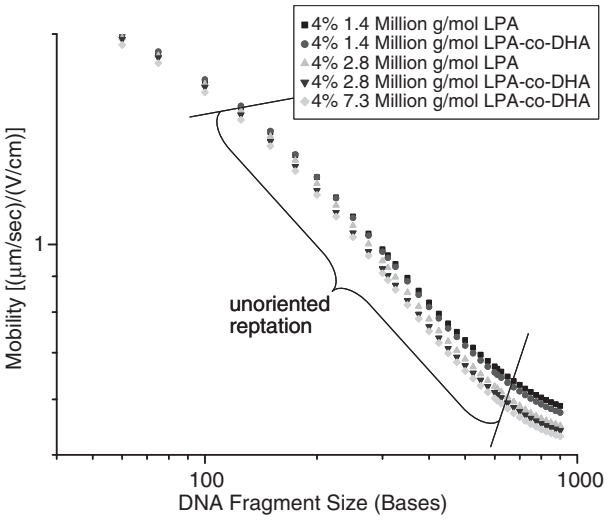
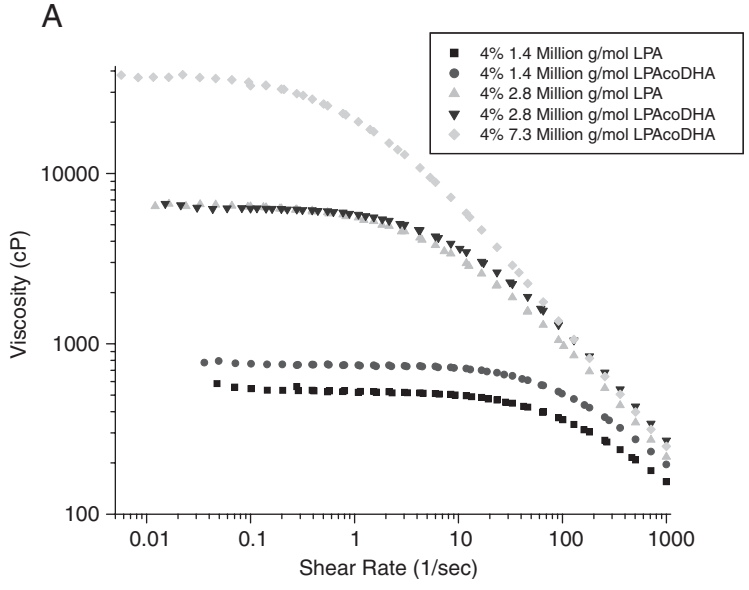


Figure 3. Mobility data obtained with a 25 bp ssDNA ET ladder comparing LPA-co-DHA copolymers and LPA homopolymers of matched molar mass. (Experimental conditions identical to those used in Fig. 1.)



B

Polymer	Loading Time
~1.4 Million g/mol LPA-co-DHA, ~0.1 mol% DHA	
2%	**10 Seconds**
3%	**30 Seconds**
4%	**1.25 Minutes**
~2.8 Million g/mol LPA-co-DHA, ~0.1 mol% DHA	
2%	**10 Seconds**
3%	**40 Seconds**
4%	**4 Minutes**
~7.3 Million g/mol LPA-co-DHA, ~0.1 mol% DHA	
2%	**15 Seconds**
3%	**3.25 Minutes**
4%	**10 Minutes**
~1.4 Million g/mol LPA	
4%	**1 Minute**
~2.8 Million g/mol LPA	
4%	**3.75 Minutes**

Figure 2. (A) Representative viscosity data *versus* shear rate for the sequencing matrices utilized in this study. (B) Comparison of the total loading times obtained on an 8 cm glass microfluidic chip using 200 psi of pressure.

behavior. Two of the DNA migration videos utilized in this study can be found in the Supporting Information.

λ-DNA is a significantly larger molecule (~50 kbp) than a typical sequencing fragment, which is in the range of 0.1–1 kbp, and is also double-stranded whereas sequencing fragments are ssDNA. However, the flexibility of the respective molecules as they migrate through the polymer matrix allows for an applicable comparison between the two. ssDNA is very flexible, with a persistence length equivalent to approximately 15 bases [35], while the structure of dsDNA makes it much stiffer, with a persistence length equivalent to approximately 150 bases [36]. This order-of-magnitude difference in their respective Kuhn lengths allows for an adequate, if simply qualitative, comparison between the fluorescently labeled dsDNA and an ssDNA sequencing fragment. ssDNA cannot be observed during electrophoresis because dyes such as YOYO cannot intercalate; and dsDNA molecules that are smaller than the genome of phage λ also cannot be observed.

As would be expected of a highly entangled polymer solution capable of sequencing long contiguous regions of DNA, the primary mechanism observed in both solutions was reptation. Reptation takes place when the polymer chains are highly entangled such that a network of pores is created with average diameters smaller than the radius of gyration of the coiled DNA molecule. In order to pass through this network the DNA must elongate in a snake-like fashion and migrate through the porous structure. The principal observed difference between the 1.4 MDa LPA and LPA-co-DHA solutions is that on average, the λ-DNA tends to remain in its elongated form for a greater period of time in the LPA-co-DHA copolymer system. Spending a greater amount of time in the elongated configuration instead of the coiled form can contribute to both the increased sequencing potential, and the slight decrease in mobility. This is because when a DNA molecule is reptating, it moves more slowly than its coiled counterpart as it interacts with the polymer network; however, because it interacts with the entangled polymer solution to a greater degree, the overall separation ability of the DNA sieving solution in improved. It should be noted that the ''inchworm chain migration dynamics'' previously observed by Chiesl *et al.* in HMPAM copolymer solutions were not seen here because the concentrations of these sequencing matrices are significantly greater, 4 *versus* 0.75% w/w, which forces the DNA molecules to reptate through a network of smaller pores [28].

4 Concluding remarks

By modifying an acrylamide-based sequencing matrix with small, randomly incorporated blocks of hydrophobic DHA monomers, we have shown that an increase in average DNA sequencing read length of up to ~10% can be achieved when compared with LPA homopolymers of equivalent molar mass. When considering the number of sequencing runs that are required to fully analyze a complex mammalian genome, an increase in read length of this degree can have a very significant impact on both the cost and time required to perform the necessary number of sequencing runs.

The HMPAM copolymer solutions are slightly more viscous than their homopolymer counterparts due to the physical cross-linking between the hydrophobic blocks, but because only ~0.1 mol% hydrophobe is necessary to achieve the improved sequencing capability and large amounts of shear thinning are observed with these sequencing matrices, the times required to load the polymer solutions into a microfluidic chip are nearly identical. The result is a copolymer solution that can consistently achieve microchip-based DNA sequencing read lengths above 550 bases, with the longest read lengths in excess of 600 bases, in a single microfluidic channel. Excellent sequencing capabilities such as these will be essential for both multiplexed genome sequencing devices as well as for single-channel microfluidic chips that will be utilized in medical and forensic analyses.

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